Radiotherapy and angiogenesis inhibition: From bench to bedside

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Chapter 2

Aim and outline

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As described in **chapter 1,** an increasing number of drugs becomes available that is directed against a specific tumor target, so-called targeted drugs. One group of targeted drugs compromises angiogenesis inhibitors. Angiogenesis is the formation of new blood vessels out of pre-existing ones. While this is a physiologic process occurring during wound healing and tissue regeneration, growing tumors also induce angiogenesis for the essential supply of oxygen and nutrients. This makes angiogenesis one of the hallmarks of cancer. Current research focuses on combining these drugs with conventional cancer therapeutics, including radiation therapy (RTx). Pre-clinical studies have shown that the combination of RTx with anti-angiogenic drugs can significantly enhance the anti-tumor efficacy. However, the optimal dosing and scheduling of both treatments is yet unknown. In addition, toxicities observed in patients have complicated translation to the clinical setting.

The aim of the research described in this thesis was twofold; 1) To study the effects of RTx on tumor angiogenesis and 2) To investigate the efficacy of different treatment schedules of the combination treatment of angiogenesis inhibition and RTx.

In order to study the combination of angiogenesis inhibition and RTx, we used several in vitro and in vivo assays, which we have described in **chapter 3**. While this chapter describes the examination of galectins, the application of other drugs works similarly, such as the angiogenesis inhibitor sunitinib. In addition, the same read out is used for monitoring radiation damage on the vasculature. This chapter also describes the use of the chorioallantoic membrane (CAM) assay, which we used for studying both in vivo angiogenesis as well as tumor growth. The CAM is the highly vascularized embryonic sac of a chicken embryo.

For the research described in this thesis we mainly used the angiogenesis inhibitor sunitinib. Sunitinib is a tyrosine kinase inhibitor (TKI) that acts by inhibiting the signal transduction of angiogenic growth factor receptors, including VEGFR1 and -2. Sunitinib is FDA and EMA approved for the treatment of metastatic renal cell carcinoma, imatinib resistant gastrointestinal stromal tumors and pancreatic neuroendocrine tumors. In addition, several (pre)clinical studies have evaluated the efficacy and feasibility of its combination with RTx, as review in **chapter 4**. This shows that in order to optimize the combination therapy and to reduce the side effects, research should firstly focus on the dosing and scheduling.

To optimize the dosing and scheduling of sunitinib with RTx, we studied the effects of both treatment strategies on angiogenesis and tumor growth in vivo, after monotherapy and the combination therapy (**chapter 5**). For this, the CAM assay was used as an in vivo angiogenesis- and tumor growth model. Here we found that both sunitinib and RTx transiently damage the CAM vasculature. In addition, RTx given before sunitinib had a greater inhibitory effect on tumor growth than given during or after sunitinib treatment. In addition, we observed that precise scheduling allowed the dose reduction of sunitinib with 50% without hampering the anti-tumor efficacy.

Although these results give insight in the importance of scheduling and dosing the two treatment modalities, the RTx was applied as a single dose. This is different from the curative

setting in patients, who usually receive multiple fractions of a low dose of RTx over a course of several weeks, so called fractionated RTx (FR RTx). Therefore, we next studied the effects of sunitinib combined with FR RTx in vitro and in vivo. Our results, described in **chapter 6**, demonstrate that both single dose and FR RTx elicit a pro-angiogenic response characterized by increased expression of multiple pro-angiogenic growth factors. In addition, we measured increased tumor perfusion with dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) and contrast enhanced ultrasound. Also cancer cell repopulation was observed in hypoxic areas of the tumors. When low dose sunitinib was applied after start of either FR or single dose RTx, this resulted in decreased tumor perfusion and hampered tumor growth.

To evaluate whether and when pro-angiogenic signaling in response to RTx also occurs in patients, we set up a clinical pilot study. In this study we selected patients with esophageal cancer, and measured pro-angiogenic factor expression before and during RTx and chemotherapy in tumor biopsies. The preliminary data of this ongoing study are described in **chapter 7**.

To get more insight in the pathways underlying the response to single dose and FR RTx we analyzed altered gene expression in cancer cells in vitro, and in irradiated tumor xenografts in vivo **(chapter 8)**. Pathway analysis revealed a type I interferon (IFN) pathway induction, which was intrinsically induced by RTx in the cancer cells. We identified the protein STING as the main activator of IFN production after RTx. The IFN pathway is generally induced by bacterial of viral infection. While it has been demonstrated before that DNA-damage after RTx can also activate STING and consequently the IFN pathway in immune cells, we now demonstrate that this activation also occurs in cancer cells.

Finally, in **chapter 9**, we discuss the novel insights obtained during our research. These insights are put in broader perspective with the current literature. In addition, we present the opportunities and future challenges of combining RTx with anti-angiogenic drugs.